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ORIGINAL ARTICLE

Streptamer versus tetramer-based selection of functional cytomegalovirus-specific T cells

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Background/Purpose: Cytomegalovirus (CMV) disease constitutes a serious complication after stem cell transplantation and has been treated by adoptive transfer of donor-derived CMV-specific $CD8^+$ T cells. CMV-specific $CD8^+$ T cells were selected by multimers, and the technologies may alter the function of these T cells. Therefore, here we evaluated the impact of multimer reagents on the function of $CD8^+$ T lymphocytes.
Methods: CMV-specific $CD8^+$ T cells were purified from the peripheral blood of donors using tetra- and streptamer technologies. The functional status of purified CMV-specific $CD8^+$ T cells was assessed by multiparametric immunophenotyping and carboxyfluorescein succinimidyl ester proliferation assays as well as by enzyme-linked immunospot assays.
Results: A similar percentage of CMV-specific $CD8^+$ T cells could be purified by both tetra- (90%) and streptamer (92%) technologies. That constitutes a 30- to 50-fold concentration of CMV-specific $CD8^+CD45RA^+CCR7^-$effector T cells. Selected cells secreted interferon-gamma and granzyme B upon stimulation with CMVpp65 peptide, thus demonstrating their functionality.
Conclusion: Our study demonstrated that both tetra- and streptamer technologies can be used to purify CMV-specific cytotoxic $CD8^+$ T cells for adoptive T-cell transfer. Both multimer

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technologies did not have any negative influence on the proliferation of selected T cells. Importantly, streptamer technology is available at good manufacturing practice level.

Introduction

Cytomegalovirus (CMV) reactivation can not only lead to severe complications, but also induce or increase graft-versus-host disease (GVHD) after allogeneic stem cell transplantation (allo-HSCT).[1–3] Prophylactic and preemptive treatment with antiviral drugs has been applied to reduce the incidence of CMV reactivation.[4,5] CMV-specific $CD8^+$ T cells were demonstrated to be of crucial importance for preventing or at least limiting CMV reactivation.[6] Therefore, adoptive transfer of virus-specific $CD8^+$ T cells was developed to prevent and treat both CMV reactivation and disease. The groups around Riddell et al[7] and Einsele et al[8] had demonstrated that virus-specific $CD8^+$ T cells could result in both an antivirus effect and a reduction of GVHD. Both groups exploited the complex and cumbersome technology of T-cell cloning, which is hardly feasible at the majority of bone marrow transplantation centers. Therefore, Cobbold et al[9] used Human Leukocyte Antigen (HLA)—peptide tetramers to purify CMV-specific $CD8^+$ T cells from CMV-seropositive donors and transferred them directly into the recipient. Recently, the novel streptamer technology has been developed for the detection and purification of antigen-specific T cells.[10] Currently, our group is investigating on CMV-specific $CD8^+$ T cells from CMV-seropositive donors using the streptamer technology. Both tetramers and streptamers can stain CMV-specific $CD8^+$ T cells.[11,12] Therefore, they might also facilitate the clinical application of adoptive CMV-specific $CD8^+$ T cells transfer regimens.[9,10] However, both multimers might also alter the functional status of CMV-specific $CD8^+$ T cells. In this study, we therefore assessed the impact of tetramers and streptamers on the function of CMV-specific $CD8^+$ T lymphocytes.

Methods

Samples from healthy donors

All samples were taken from HLA-$A2^+$ healthy donors after informed consent was obtained in accordance with the Declaration of Helsinki. The study was approved by the local Ethical Committee (HV-2009-0004). Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll—Biocoll separation solution (Biochrom, Berlin, Germany) density gradient centrifugation from EDTA (Delta-Pharma, Pfullingen, Germany) anticoagulated blood buffy coat preparations from healthy donors. The viability of PBMCs was always >95%, as determined by Trypan blue staining (Trypan Blue Solution 0.4%, Sigma-Aldrich, Munich, Germany). Viable cells were quantified using a Neubauer chamber (Zeiss, Oberkochen, Germany). For cellular assays, Ficoll-separated PBMCs were tested freshly or cryopreserved in RPMI 1640 containing 20% human AB serum and 10% dimethyl sulfoxide (Merck, Darmstadt, Germany), and stored in liquid nitrogen.

Flow cytometry for multiparametric immunophenotyping

To evaluate the percentage of the CMV-specific $CD8^+$ T cells, samples were analyzed by flow cytometry. $CD8^+$ T cells were detected using peridinin chlorophyll (PerCP)-labeled anti-CD8 antibodies. CMV-specific $CD8^+$ T cells were detected by using the conjugate of CMVpp65 peptide—Major Histocompatibility Complex (MHC) class I complex with Strep-Tactin-PE. HLA-A2*0201/CMV peptide streptamer*PE was specifically synthesized at IBA GmbH (Göttingen, Germany). For staining, the cells were incubated with the HLA-A2*0201/CMV peptide streptamer*PE for 45 minutes at 4°C in the dark. CMV—MHC complex was used at a concentration of 0.2 μg per 1×10^6 cells in 100 μL per test. For multiparametric analysis, anti-CD8*PerCP, anti-CCR7*APC, anti-CD45RA*FITC, anti-CD69*APC, anti-CD107a*FITC, anti-CD137*APC, and anti-CD28*FITC (BD, Heidelberg, Germany) antibodies were added at 4°C for 20 minutes in the dark. After washing twice with 1 × phosphate buffered saline (PBS), stained cells were analyzed by flow cytometry (FACSAria, BD). In all cases, at least 100,000 events were collected for analysis. Each sample was run with an appropriate isotype control, and this was used to define the stained cells. Analysis was performed on tightly gated lymphocytes to exclude dead cells and debris.

Isolation of CMV-specific T cells with streptamer magnetic beads

The procedure was optimized to isolate CMV-specific $CD8^+$ T cells from 2×10^7 PBMCs and performed according to the manufacturer's instructions. First, a magnetic streptamer complex was used to label CMV-specific T cells according to their antigen specificity. Thereafter bead-labeled cells were separated from nonspecific cells by a magnetic field. Later, D-biotin was added to the purified CMV-specific T cells. Briefly, this process was divided into three steps.

In the first step, 50 μL streptamer magnetic beads were incubated with 8 μL CMV—MHC and 90 μL IS buffer (IBA GmbH) overnight at 4°C in the dark. Then 1 mL IS buffer was added to MHC/streptamer magnetic beads solution and loaded on an medium scale (MS) column in the magnetic field to wash unbound MHC and streptamer magnetic beads away. Therefore, the MS column was taken outside of the magnetic field and the retained beads were firmly flushed out by 250 μL IS buffer using the plunger supplied with the column. A total of 2×10^7 cells were gently mixed with the 250 μL MHC/streptamer magnetic beads solution and incubated for 45 minutes at 4°C in the dark. These cells were washed and suspended in 1 mL IS buffer for magnetic separation.

In the second step, the MS column was placed in the magnetic field and prepared by rinsing with 3 mL IS buffer, and the suspended cells above were applied onto the column, allowing the unlabeled cells to pass through. After washing three times with 1 mL IS buffer, the MS column was taken outside of the magnetic field and retained cells were eluted into a fresh vial by 6 mL IS buffer using the plunger supplied with the column. Then, the positive cell fraction was collected for the next step.

In the third and last step, the dissociation of the MHC/streptamer magnetic beads from the cells was performed using D-biotin. The fraction of positive cells was centrifuged and resuspended in 2 mL IS buffer containing 2 mM D-biotin and incubated on ice for 20 minutes in the dark twice. Thereafter, the cells were washed with 5 mL IS buffer four times. Eventually, these cells were collected for further analysis.

Selection of CMV-specific T cells by tetramer magnetic beads

After washing with magnetic cell separation (MACS) buffer (Miltenyi Biotec, Bergisch Gladbach, Germany), HLA-A2$^+$ cells from peripheral blood samples of healthy donors were resuspended in 200 μL MACS buffer and incubated with HLA-A2$^+$ tetramer hCMVpp65 495—503 (NLVPMVATV) *PE at a concentration of 10 μL per 10^7 cells for 40 minutes at room temperature in the dark. Cells were washed twice and resuspended in MACS buffer, and anti-PE MicroBeads (Miltenyi Biotec) were added at a concentration of 20 μL per 10^7 cells for 15 minutes at 4°C in the dark. Then, these cells were washed and resuspended in 1 mL MACS buffer for magnetic separation. The MS column was placed in the magnetic field and prepared by rinsing with 500 μL MACS buffer, and the resuspended cells above were applied onto the column, allowing the unlabeled cells to pass through. After washing with 3 × 500 μL MACS buffer, the MS column was taken outside of the magnetic field. Retained cells were eluted into a fresh vial using 1 mL MACS buffer and the plunger supplied with the column. The positive fraction was collected for further analysis.

Carboxyfluorescein succinimidyl ester proliferation assay

After isolation of CMV-specific CD8$^+$ T cells, CD8$^-$ cells serving as antigen presenting cell (APCs) were irradiated with 30 Gy and incubated with 20 μg/mL CMV peptide and 2.5 μg/mL β_2-microglobulin in 1 mL medium supplemented with 10% AB medium for 2 hours at 37°C. CMV-specific CD8$^+$ T cells (1 × 10^6 cells/mL) were labeled with the vital dye carboxyfluorescein succinimidyl ester (Invitrogen Gibco, Grand Island, NY, USA) at a final concentration of 0.5 μM for 15 minutes at 37°C and cocultured with CD8$^-$ APCs. After coincubation, mixed lymphocyte peptide culture (MLPC) was supplemented with 2.5 ng/ml IL-2 and 20 ng/mL IL-7 (Sigma, St. Louis, MO, USA) on day+1. After 8 days of culture, proliferation of CMV-specific CD8$^+$ T cells was evaluated by fluorescence activated cell sorting (FACS) analysis as described above.

Enzyme-linked immunospot assay

Enzyme-linked immunospot (ELISPOT) assays for interferon (IFN)-gamma and granzyme B were performed as previously described.[13] A total of 4 × 10^3 presensitized CD8$^+$ T lymphocytes and 1 × 10^4 target cells [CMVpp65 or human epidermal growth factor receptor-2 (HER 2) peptide pulsed T2 cells] were added to each well. Spots were evaluated using an ELISPOT reader (C.T.L. Ltd, Bonn, Germany). Results are presented as an average of duplicate cultures.

Results

Frequency of CMV-specific CD8$^+$ T cells in healthy donors

In order to select samples with high frequency of CMV-specific CD8$^+$ T cells, we analyzed 30 peripheral blood samples from HLA-A2$^+$/CMV-seropositive healthy donors. All the samples were analyzed by FACS staining using CMV/HLA-A2$^+$ CMVpp65 495—503 (NLVPMVATV) streptamers. Samples from 10 HLA-A2$^+$ but CMV-seronegative healthy donors were tested as controls, and no CMV-specific CD8$^+$ T cells were detected (data not shown). In all 30 HLA-A2$^+$/CMV-seropositive healthy donors, we were able to detect CMV-specific CD8$^+$ T cells. The frequency of CD8$^+$ T cells specifically recognizing the HLA-A2-restricted CMV epitope ranged from 0.15% to 4.01% when compared to all CD8$^+$ T cells. In 16 (53.3%) of 30 HLA-A2$^+$/CMV-seropositive healthy donors, frequencies of more than 0.5% for CMV-specific CD8$^+$ T cells were detected when compared to all CD8$^+$ T cells; in 11 healthy donors we detected frequencies over 1.0% of all CD8$^+$ T cells (Table 1). These samples were used for further analysis.

Purity of CMV-specific CD8$^+$ T cells separated by CMV streptamer technology

To evaluate the purity of the enriched CMV-specific CD8$^+$ T cells separated by CMV streptamer magnetic beads, the positive fraction was stained and analyzed by flow cytometry. After separation of PBMCs, CMV-specific CD8$^+$ T cells were >90% of the total CD8$^+$ T-cell population; a 35—50-fold up-concentration of CMV-specific CD8$^+$ T cells could be achieved, when compared to the total CD8$^+$ T-cell population (Fig. 1).

Frequency of CMV-specific CD8$^+$ T cells purified by tetramers

In order to evaluate the purity of the CMV-specific CD8$^+$ T cells selected by tetramers, the positive fraction was directly analyzed by flow cytometry. After selection of PBMCs, CMV-specific CD8$^+$ T cells accounted for more than 90% of the total CD8$^+$ T-cell population. Also, here a 30—50-fold increase in purity could be achieved for CMV-specific CD8$^+$ T cells when compared to the total CD8$^+$ T-cell population (Fig. 2).

Table 1 Percentage of $CD8^+$ T cells specific for CMV in $HLA\text{-}A2^+$/CMV-seropositive healthy volunteers.

No. of healthy volunteers	CMV-specific $CD8^+$ T cells/$CD8^+$ T cells (%)
1	3.16
2	1.85
3	0.15
4	0.27
5	1.16
6	1.53
7	0.17
8	0.35
9	1.46
10	0.23
11	0.31
12	0.30
13	0.25
14	0.80
15	0.32
16	0.21
17	0.46
18	0.88
19	0.17
20	1.35
21	1.50
22	3.14
23	0.24
24	2.84
25	0.16
26	0.84
27	0.50
28	0.82
29	4.01
30	1.65

CMV = cytomegalovirus.

Immunophenotyping of CMV-specific $CD8^+$ T cells

In order to investigate the potential functional status of CMV-specific $CD8^+$ T cells purified by streptamers and tetramers, samples of $HLA\text{-}A2^+$/CMV-seropositive healthy donors before and after separation were identified by staining for phenotypic markers. CMV-specific $CD8^+$ T cells before and after separation showed a similar CD antigen expression (Figs. 1 and 2), with an exception of the increase in CD28 expression of CMV-specific $CD8^+$ T cells after streptamer selection. CMV-specific $CD8^+$ T cells stained by streptamer and tetramer showed a similar frequency of $CD45RA^+CCR7^-$ effector T cells. Furthermore CMV-specific $CD8^+$ T cells, after separation, proved to be almost 100% $CD45RA^+$ T cells, mostly (80—94%) $CCR7^-$eff. T cells (Figs. 1 and 2).

Figs. 1—4 show data from the same sample. Similar data were obtained from two further samples.

Proliferation of CMV-specific $CD8^+$ T cells purified by tetramer and streptamer technologies

Potential functional characteristics of CMV-specific $CD8^+$ T cells purified by streptamer and tetramer were further analyzed by proliferation assays. MLPC followed by multimer (streptamer and tetramer) staining was used to detect proliferation of CMV-specific $CD8^+$ T-cell populations. The results showed that 44.1% of CMV-specific $CD8^+$ T cells purified by streptamer proliferated after 8 days of culture, and, for the CMV-specific $CD8^+$ T cells purified by tetramer, the frequency was similar (42.9%) (Fig. 3).

ELISPOT assays for IFN-gamma and granzyme B

Activation of lytic potential of CMVpp65-specific $CD8^+$ T cells was assessed by ELISPOT. As shown in combined representative results from three independent experiments, both

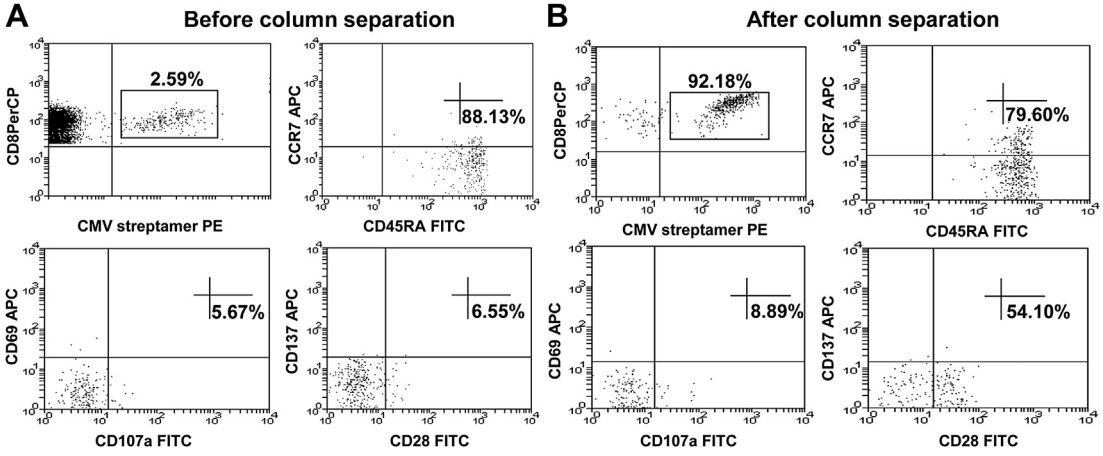

Figure 1 Isolation and immunophenotyping of CMV-specific $CD8^+$ T cells by streptamers. The dot plots show the percentage of $HLA\text{-}A2^+$/CMV streptamer (PE)-positive $CD8^+$ T lymphocytes (A) before and (B) after separation. A 35—50-fold increase in the up-concentration percentage of $HLA\text{-}A2^+$/CMV streptamer (PE)-positive $CD8^+$ T lymphocytes could be observed after MACS separation. CMV streptamer-specific $CD8^+$ T cells were further characterized by FACS analysis, demonstrating the existence of CMV-streptamer$^+CD8^+CD45RA^+CCR7^-$ T cells in the peripheral blood of healthy donors, thus indicating the effector T-cell status of these cells. Panel A (before separation) and Panel B (after separation) show a similar immunophenotype of the CMV-specific T cells. This experiment has been repeated several times with similar results. CMV = cytomegalovirus.

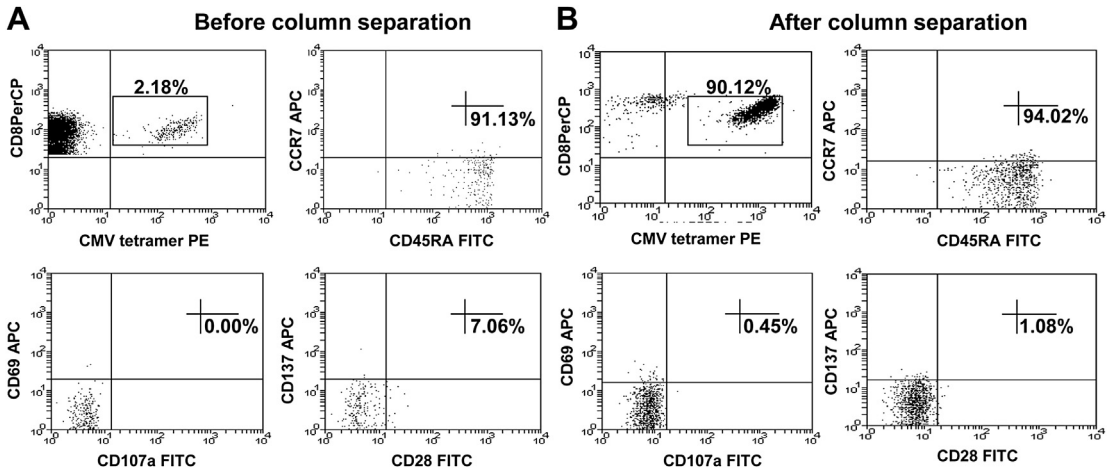

Figure 2 Isolation and immunophenotyping of CMV-specific $CD8^+$ T cells by tetramers. The dot plots show the percentage of HLA-$A2^+$/CMV tetramer (PE)-positive $CD8^+$ T lymphocytes (A) before and (B) after separation. For subpopulations of $CD8^+$ T cells, a 30—50-fold increase in the percentage of HLA-A2/CMV tetramer (PE)-positive $CD8^+$ T lymphocytes could be observed after MACS separation. CMV tetramer-specific $CD8^+$ T cells were further characterized by FACS analysis, demonstrating the existence of $CD8^+$CMV-tetramer$^+$$CD45RA^+$$CCR7^-$ T cells in the peripheral blood of healthy donors, thus indicating the effector T-cell status of these cells. Panel A (before separation) and Panel B (after separation) expressed a similar immunophenotype. This experiment has been repeated several times with similar results. CMV = cytomegalovirus.

IFN-gamma (Fig. 4A) and granzyme B (Fig. 4B) were secreted by either streptamer- or tetramer-selected CMVpp65-specific $CD8^+$ T cells upon stimulation with the autologous $CD8^-$ APCs pulsed with CMVpp65 peptide. CMVpp65-specific $CD8^+$ T lymphocytes selected by streptamer beads proved to be very effective (Fig. 4).

Discussion

Reactivation of CMV constitutes a severe and potentially life-threatening complication after allo-HSCT. Because of immunosuppression after allo-HSCT, the latent virus can reactivate, leading to CMV disease. Manifestations of CMV disease are pneumonia, colitis, and encephalitis.[1—3] Prior to the introduction of antiviral therapy, the frequency of CMV reactivation was up to 80% of CMV-seropositive allogeneic recipients and 40% of the patients developed CMV disease.[3,14] The use of antiviral drugs decreased mortality due to CMV disease after allo-HSCT dramatically.[15,16] However, antiviral drugs might cause substantial myelotoxicity, resulting in neutropenia and bacterial as well as fungal infections. CMV reactivation might trigger GVHD.

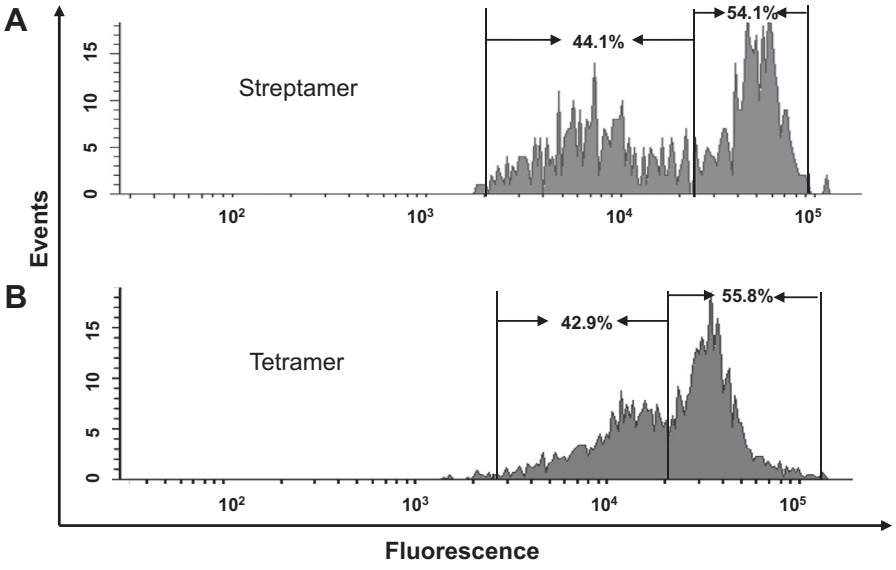

Figure 3 Proliferation characteristics of CMV-specific $CD8^+$ T cells purified by streptamers and tetramers. (A) The purification of CMV-specific $CD8^+$ T cells selected by streptamer resulted in a frequency of 44.1% proliferating cells. (B) 42.9% of CMV-specific $CD8^+$ T cells purified by tetramers were proliferating after 8 days of culture. The results indicated that there was a significant proliferation when streptamer- and tetramer-selected CMV-specific $CD8^+$ T cells were cultured. This experiment has been repeated two times with similar results. CMV = cytomegalovirus.

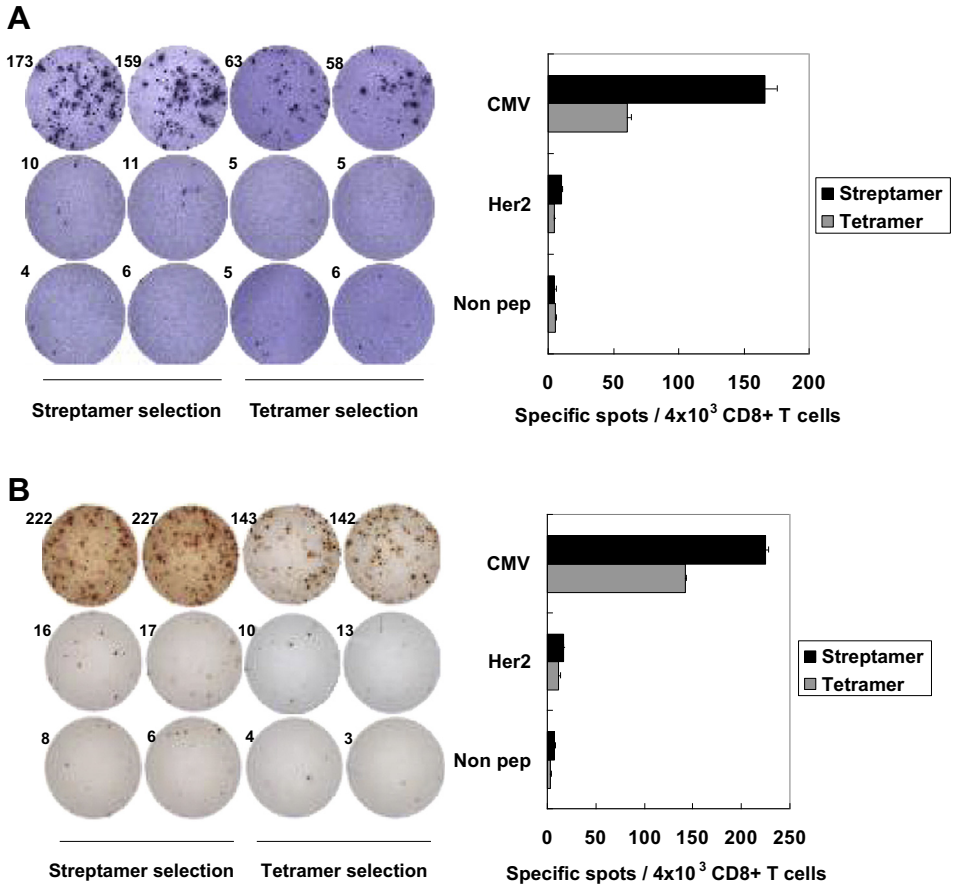

Figure 4 Specific reactivity of $CD8^+$ T lymphocytes to CMVpp65 peptide assessed by ELISPOT. CMV-specific $CD8^+$ T cells were isolated by both streptamers and tetramers from a healthy donor and subjected to one round of stimulation with irradiated and CMVpp65 peptide-loaded $CD8^-$ APCs. Their potential to secrete (A) IFN-gamma and (B) granzyme B was measured by ELISPOT assays employing T2 cells pulsed with CMVpp65 peptide, an irrelevant HLA-A2-restricted HER 2-derived peptide to demonstrate the CMV specificity of the $CD8^+$ T cells. T2 cells without loading of any peptide were used as negative control. The figure shows one representative experiment out of three. Error bars indicate the standard deviation. CMV = cytomegalovirus; ELISPOT = enzyme-linked immunospot; HER 2 = human epidermal growth factor receptor-2; IFN = interferon.

Moreover, CMV can become resistant to antiviral drugs,[17] limiting the use of such drugs.

This dilemma has been alleviated by the use of adoptive transfer of CMV-specific $CD8^+$ T cells. Two strategies have been described to select CMV-specific $CD8^+$ T cells: First, CMV-specific $CD8^+$ T-cell clones were cultured from the transplant donors and infused to the patients after HSCT[7,8,18] or even solid organ transplantation.[19] Second, CMV-specific $CD8^+$ T cells can be directly selected from the blood of transplant donors and infused into the patients without *ex vivo* manipulation.[9] CMV-specific $CD8^+$ T-cell cloning is expensive and demands a cumbersome and skillful technique, limiting its use to only few specialized centers of expertise. Therefore, the approach of the selection of CMV-specific $CD8^+$ T cells directly from the blood of stem cell transplant donors was recommended by Cobbold and coworkers[9] because of its rather simple technique and high efficacy.

Our study showed that in all 30 HLA-$A2^+$/CMV-seropositive healthy donors, the frequency of CMV-specific $CD8^+$ T cells, when compared to all $CD8^+$ T cells, ranged from 0.15% to 4.01%. In 11 of 30 HLA-$A2^+$/CMV-seropositive healthy donors, more than 1.0% of all $CD8^+$ T cells were specific for CMVpp65. Gillespie et al[20] also reported that CMV-specific $CD8^+$ T cells range from 0.5% to 4% of the $CD8^+$ T-cell pool. The data of our present study indicate that we could select a sufficient number of CMV-specific $CD8^+$ T cells directly from the blood of healthy donors without *ex vivo* manipulation.

With the help of multimer technologies one can identify and select CMV-specific $CD8^+$ T cells. Therefore, multimers constitute a benefit for the clinical application of adoptive CMV-specific $CD8^+$ T-cell transfer.[9,10,21] However, multimer technologies may have some adverse influence on these T cells. Although Cobbold et al[9] reported that use of HLA—peptide tetramer has a considerable potential for antigen-specific $CD8^+$ T-cell therapy, Neudorfer et al stated that regarding the selection of antigen-specific $CD8^+$ T cells for clinical application, the reversible streptamer technique might be favorable when compared to the tetramer method. Therefore, the approach for the selection of CMV-specific $CD8^+$ T cells directly from the blood of healthy donors by both multimer technologies (tetramer and streptamer) was assessed in the present study.

A 30—50-fold increase in purity of specific CD8+ T cells could be achieved through the selection by both tetramer and streptamer technologies when compared to total $CD8^+$ T-cell population. The purity that we could achieve in this study was more than 90% for both methods. The difference of both multimer technologies is that CMV-specific $CD8^+$ T-cell selection by streptamer technology was followed by a dissociation of streptamer with D-biotin, thus avoiding potential side effects resulting from the multimer reagents and allowing the selection of antigen-specific $CD8^+$ T cells with preserved, naïve function.[10] Streptamer-coated beads can be detached following selection, which is in accordance with the guidelines. Therefore, bead-conjugated streptamers constitute a further development of good manufacturing practice (GMP) of the currently used tetramer-based selection methods.[9—11]

The functional status of CMV-specific $CD8^+$ T cells selected by streptamers and tetramers was identified by the staining of phenotypic markers. On the basis of CD45RA and CCR7 expression, $CD8^+$ T cells can be dissected into four groups: naïve (N: $CD45RA^+CCR7^+$), effector (E: $CD45RA^+CCR7^-$), central memory (CM: $CD45RA^-CCR7^+$), and effector memory (EM: $CD45RA^-CCR7^-$) T cells.[22] Different states of $CD8^+$ T-cell activation imply different functional and immunophenotypic characteristics. We performed staining with anti-CD69 as a marker of activation of T cells as well as with CD107a as a marker of intracellular granzyme B. CD137 was used as another activation marker, and staining against CD28 was performed to distinguish early and late effector memory cells.[22] In this study, we found that CMV-specific $CD8^+$ T cells identified by tetramer and streptamer technologies before and after separation were consistently $CD8^+$CMV-multimer$^+CD45RA^+CCR7^-$ T cells. The ratio of CMV-specific $CD8^+$ T cells was more than 90% of all $CD8^+$ T cells after selection. Our results indicate that most selected CMV-specific $CD8^+$ T cells have an effector T-cell immunophenotype, and the functional status of $CD8^+$ T cells might be preserved. One exception was the increase in CD28 expression of CMV-specific $CD8^+$ T cells after streptamer selection. This might indicate a further positive activation of these specific T cells.[22]

Regarding the proliferation of CMV-specific $CD8^+$ T cells, the results showed that 44.1% of these T cells purified by streptamer proliferated after 8 days of culture, and for the T cells purified by tetramer, the frequency was 42.9%. The results showed a significant proliferation when CMV-specific $CD8^+$ T cells selected by both streptamer and tetramer technologies were cultured. However, the functional status of tetramer-selected CMV-specific $CD8^+$ T cells might be altered by the persistent binding of tetramer reagents. This might also be the reason for the somewhat altered pattern of the curve in the Carboxyfluorescein succinimidyl ester (CSFE) staining (Fig. 3). The functional activity of tetramer-labeled CMV-specific $CD8^+$ T cells could also be hampered by the persistence of TCR—MHC interactions resulting from tetramer and subsequently induced signaling events.[23,24] In this study, we could clearly demonstrate, by ELISPOT assays, that streptamer-selected CMV-specific $CD8^+$ T cells are active (secretion of IFN-gamma) and that they are cytotoxic (secretion of granzyme B). As streptamer reagents could be detached from the isolated CMV-specific $CD8^+$ T cells, several side effects caused by reagents might be avoided, including T-cell anergy, harm for clinical *in vivo* application, immune responses directly against the reagents, and loss of the capacity of the transferred T cells to migrate *in vivo*. This indicates that the streptamer technology might constitute a step further in the development of multimers for adoptive T-cell therapy.[25]

In summary, the multimer technology comprising both streptamers and tetramers constitutes an interesting novel tool for further adoptive transfer of CMV-specific $CD8^+$ T cells. Moreover, the streptamer technology is available at GMP level, thus allowing the ("reversible") selection of CMV-specific $CD8^+$ T cells while preserving their function.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contribution

WX and XX did the acquisition and analysis of the data. AS and MS substantially contributed to conception, design, and interpretation of the data. MF, PH, MS, and CB have been involved in drafting the manuscript or revising it critically. MS has given the final approval of the version to be published.

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